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06 FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE, GENBANK' ENTERED AT 15:03:41 ON
SEP 2001
L1 155524 S GENE?(P)REPLAC?
L2 538 S L1 (P)MRNA (P)CAP?
L3 212 S L2 (P)5
L4 24 S L3 (P)TRANSFORM?
L5 7 DUPLICATE REMOVE L4 (17 DUPLICATES REMOVED)

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L7 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2001 ACS
AN 2000:589929 CAPLUS
DN 133:172151

TI Screening for inhibitors of mRNA cap formation for use as antimycotics
using host cells with fungal or mammalian capping enzymology

IN Shuman, Stewart

PA USA

SO U.S., 62 pp.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6107040	A	20000822	US 1998-188579	19981109
	US 6232070	B1	20010515	US 1999-315444	19990520
	WO 2000063433	A1	20001026	WO 1999-US26520	19991109
	W: AU, CA, JP, MX				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	EP 1127164	A1	20010829	EP 1999-962733	19991109
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRAI US 1998-188579 A2 19981109
US 1999-315444 A 19990520
WO 1999-US26520 W 19991109

AB This invention provides methods for the discovery of mols. that target an essential aspect of eukaryotic gene expression-the formation of the mRNA 5' cap m7GpppN. An underlying principle of this invention is the use of a different strains of a test organism that differ only in the compn. or source of the essential cap-forming enzymes. The invention provides isogenic yeast strains that derive all their capping activities from fungal sources vs. mammalian sources. These strains form the basis of a differential growth inhibition assay to identify mols. that specifically target the fungal capping app. This invention also provides a method to screen in vitro for mols. that inhibit

fungal RNA triphosphatase, an essential enzyme that catalyzes the first of three steps in cap synthesis. The ability of human capping activities to replace those of *Saccharomyces cerevisiae* is demonstrated.

RE.CNT 2

RE

- (1) Ho; Molecular and Cellular Biology 1998, V18(9), P5189 CAPLUS
- (2) Yue; Mammalian capping enzyme complements mutant *Saccharomyces cerevisiae* lacking mRNA guanylytransferase and selectively binds the elongating form of RNA polymerase 2 1997, V94, P12898 CAPLUS

L7 ANSWER 4 OF 4 MEDLINE DUPLICATE 2
AN 1999278431 MEDLINE
DN 99278431 PubMed ID: 10347220
TI Characterization of human, *Schizosaccharomyces pombe*, and *Candida albicans*
mRNA cap methyltransferases and complete replacement of the yeast capping apparatus by mammalian enzymes.
AU Saha N; Schwer B; Shuman S
CS Molecular Biology Program, Sloan-Kettering Institute, New York, New York 10021, USA.

NC GM52470 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jun 4) 274 (23) 16553-62.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199907
ED Entered STN: 19990714
Last Updated on STN: 19990714
Entered Medline: 19990701
AB Human and fission yeast cDNAs encoding **mRNA** (guanine-N7) methyltransferase were identified based on similarity of the human (Hcm1p; 476 amino acids) and *Schizosaccharomyces pombe* (Pcm1p; 389 amino acids) polypeptides to the **cap** methyltransferase of *Saccharomyces cerevisiae* (Abd1p). Expression of PCM1 or HCM1 in *S. cerevisiae* complemented the lethal phenotype resulting from deletion of the ABD1 **gene**, as did expression of the NH₂-terminal deletion mutants PCM1(94-389) and HCM1(121-476). The CCM1 **gene** encoding *Candida albicans* **cap** methyltransferase (Ccmlp; 474 amino acids) was isolated from a *C. albicans* genomic library by selection for complementation of the conditional growth phenotype of *S. cerevisiae* abd1-ts mutants. Human **cap** methyltransferase was expressed in bacteria, purified, and characterized. Recombinant Hcm1p catalyzed quantitative S-adenosylmethionine-dependent conversion of GpppA-**capped** poly(A) to m⁷GpppA-**capped** poly(A). We identified by alanine-scanning mutagenesis eight amino acids (Asp-203, Gly-207, Asp-211, Asp-227, Arg-239, Tyr-289, Phe-291, and Phe-354) that are essential for human **cap** methyltransferase function *in vivo*. All eight residues are conserved in other cellular **cap** methyltransferases. Five of the mutant human proteins (D203A, R239A, Y289A, F291A, and F354A) were expressed in bacteria and found to be defective in **cap** methylation *in vitro*. Concordance of mutational effects on Hcm1p, Abd1p, and vaccinia **capping** enzyme underscores a conserved structural basis for **cap** methylation in DNA viruses, yeast, and metazoans. This is in contrast to the structural and mechanistic divergence of the RNA triphosphatase components of the yeast and metazoan **capping** systems. Nevertheless, we demonstrate that the entire three-component yeast **capping** apparatus, consisting of RNA 5'-triphosphatase (Cet1p), RNA guanylyltransferase (Ceg1p), and Abd1p could be **replaced** *in vivo* by the two-component mammalian apparatus consisting of a bifunctional triphosphatase-guanylyltransferase Mcelp and the methyltransferase Hcm1(121-476)p. Isogenic yeast strains with **fungal** versus mammalian **capping** systems should facilitate rational screens for antifungal drugs that target **cap** formation *in vivo*.

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USPT	l2 same 5	36	<u>L3</u>
USPT	l1 same mRNA same cap\$	92	<u>L2</u>
USPT	gene same replac\$	8727	<u>L1</u>

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L3: Entry 7 of 36

File: USPT

Jan 16, 2001

DOCUMENT-IDENTIFIER: US 6174669 B1

TITLE: Method for making full-length cDNA libraries

BSPR:

As conventional methods for synthesizing full-length cDNAs, the following methods can be mentioned; the method utilizing a Cap binding protein of yeast or Hela cells for labeling the 5' Cap site (I. Edery et al., "An Efficient Strategy To Isolate Full-length cDNAs Based on an mRNA Cap Retention Procedure (CAPture)", MCB, 15, 3363-3371, 1995); the method where phosphates of incomplete cDNAs without 5' Cap are removed by using alkaline phosphatase and then the whole cDNAs are treated with de-capping enzyme of tobacco mosaic virus so that only the full-length cDNAs have phosphates (K. Maruyama et al., "Oligo-capping: a simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides", Gene, 138, 171-174, 1995., S. Kato et al., "Construction of a human full-length cDNA bank", Gene, 150, 243-250, 1995) and the like.